Bioinformatics

CS300

Genome Sequencing and Assembly

Chapter 8

Week10, Deck 1

Fall 2022

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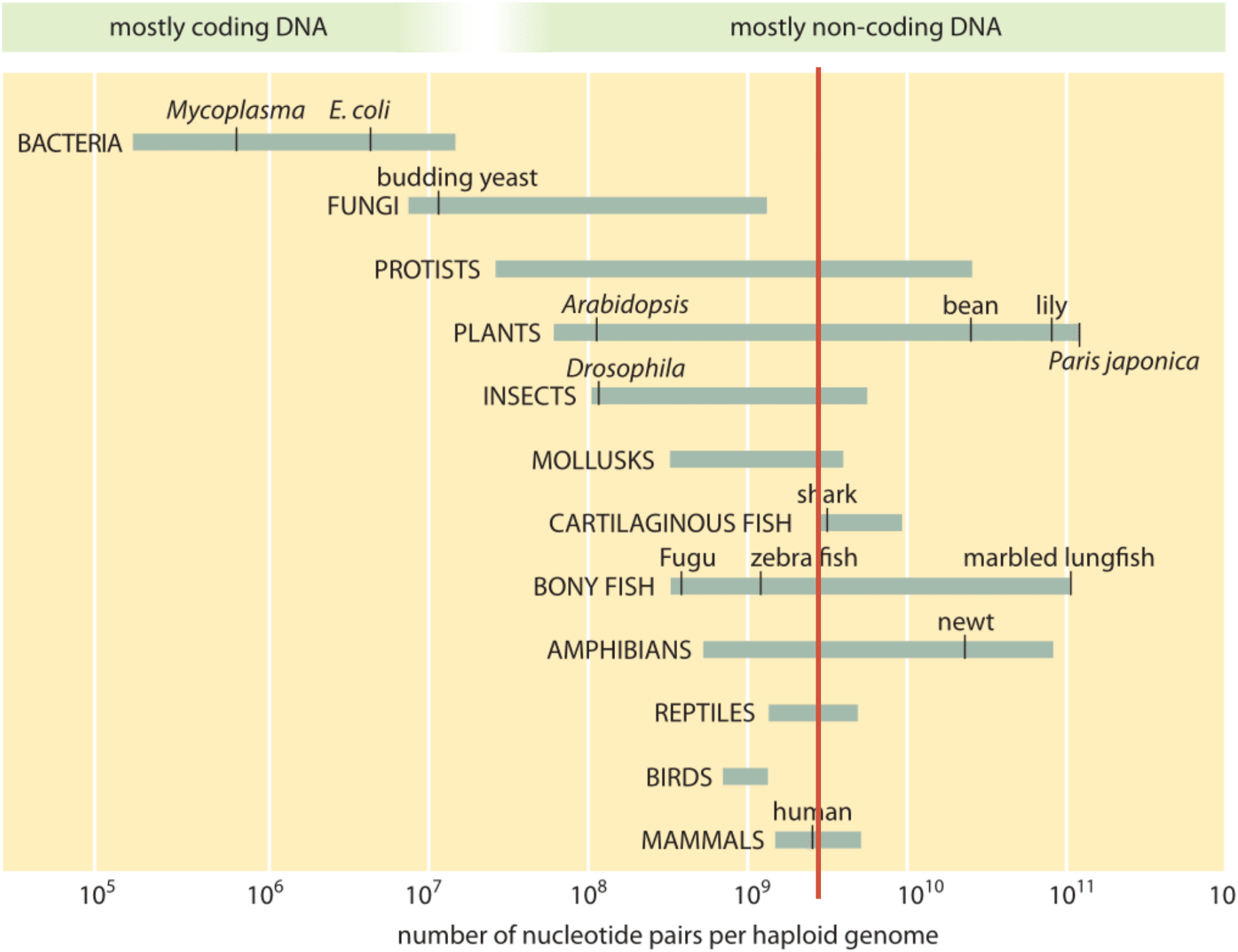
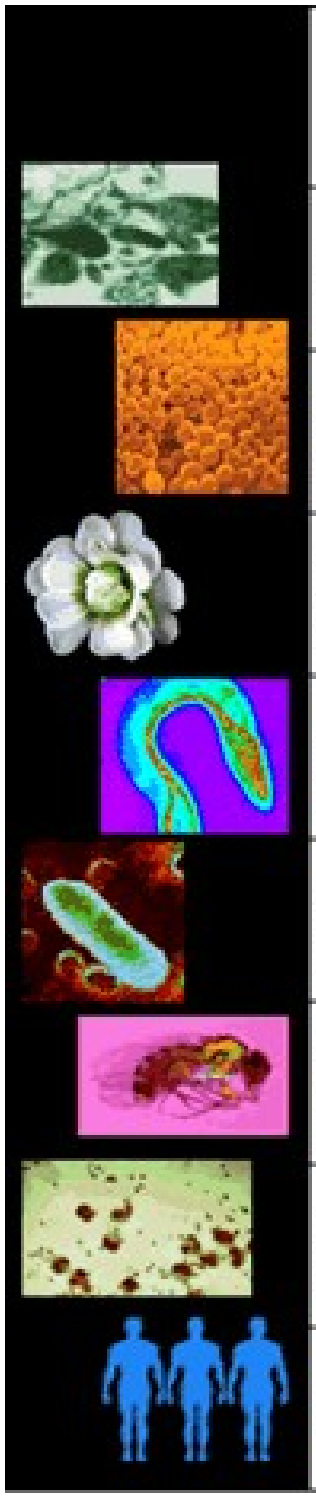
What is a Genome?

- An organism's complete set of DNA, including all of its genes, regulatory regions, non-coding regions, etc.
- An organism's complete set of genetic instructions


mostly coding DNA
mostly non-coding DNA
Mycoplasma
E. coli
BACTERIA
budding yeast
FUNGI
PROTISTS
Arabidopsis
bean
lily
PLANTS
Paris japonica
Drosophila
INSECTS
MOLLUSKS
shark
CARTILAGINOUS FISH
Fugu
zebra fish
marbled lungfish
BONY FISH
newt
AMPHIBIANS
REPTILES
BIRDS
human
MAMMALS
10^5
10^6
10^7
10^8
10^9
10^{10}
10^{11}
10^{12}
number of nucleotide pairs per haploid genome

What Is In a Genome?



Organism	Number of genes in the genome
Myscoplasma genitalium	517
Saccharomyces cerevisiae	6,275
Arabidopsis thaliana	~ 20,000
Caenorhabditis elegans	19,099
Haemophilus influenzae	1,743
Drosophila melanogaster	13,601
Neisseria meningitdis	2,158
Homo sapiens	20,000–25,000

Genome Projects

- Goals:
  - Determine complete genome sequence of an organism
  - Annotate (*exhibit*) protein-coding genes and other important genome-encoded features

Genome Projects

- Projects:
  - Lots and lots of genome projects in progress or completed

Genome Information by organism

Search by organism | Clear

Download Reports from FTP site

Overview [30649] | Eukaryotes [4874] | Prokaryotes [118997] | Viruses [7497] | Plasmids [10401] | Organelles [10835]

Download selected records

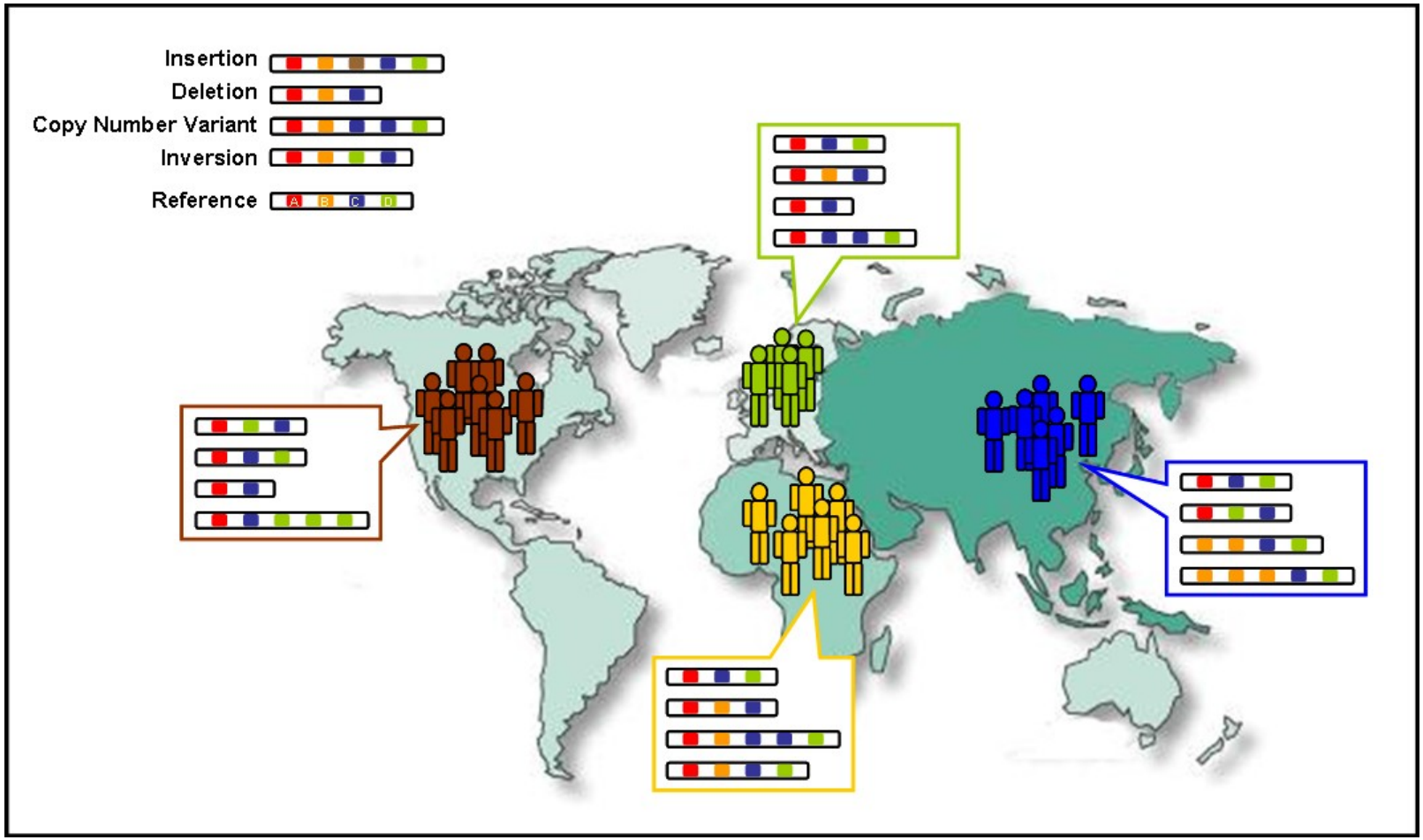
Items 1 - 100 of 30649 << First < Prev Page 1 of 307 Next > Last >>

Organism/Name	Kingdom	Group	SubGroup	Size (Mb)	Chr	Organelles	Plasmids	Assemblies
	All	All	All					
'Chrysanthemum coronarium' phytoplasma	Bacteria	Terrabacteria group	Tenericutes	0.739592	-	-	-	1
'Echinacea purpurea' witches'-broom phytoplasma	Bacteria	Terrabacteria group	Tenericutes	0.545427	-	-	-	1
'Osedax' symbiont bacterium Rs2_46_30_T18	Bacteria	unclassified Bacteria	unclassified Bacteria (miscellaneous)	4.02183	-	-	-	1
Abaca bunchy top virus	Viruses	ssDNA viruses	Nanoviridae	0.006422	6	-	-	1
Abalone herpesvirus Victoria/AUS/2009	Viruses	dsDNA viruses, no RNA stage	unclassified	0.211518	1	-	-	1
Abalone shriveling syndrome-associated virus	Viruses	dsDNA viruses, no RNA stage	unclassified	0.034952	1	-	-	1
Abelson murine leukemia virus	Viruses	Retro-transcribing viruses	Retroviridae	0.005894	1	-	-	1

https://www.ncbi.nlm.nih.gov/genome/browse/

Genome Projects

- Contrast genetic material of populations to determine ancestry



https://en.wikipedia.org/wiki/1000_Genomes_Project#Human_genetic_variation

Genome Projects: Data

- Annotations: gene locations for protein products in sequences.

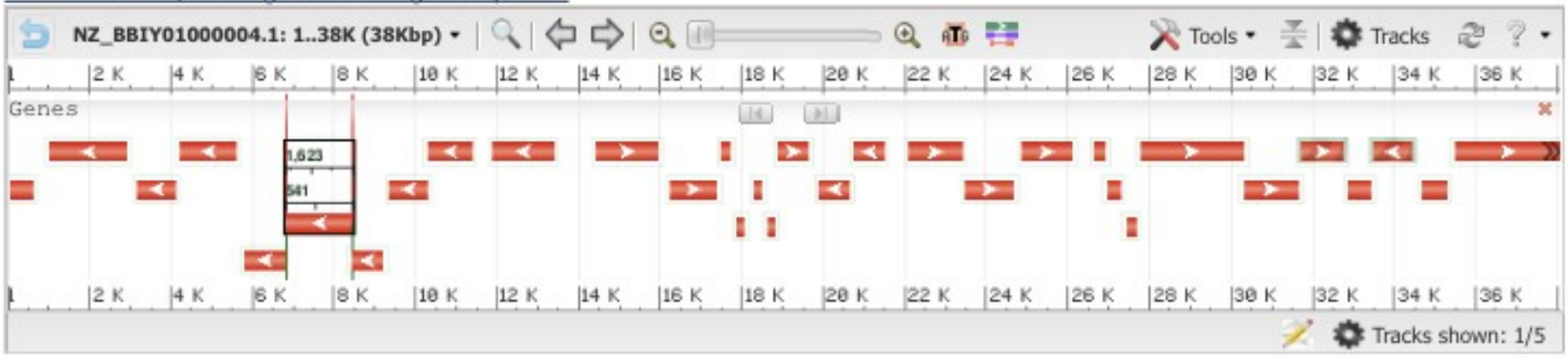
Genome Assembly Annotation

Type	Name	RefSeq	INSDC	Size (Mb)	GC%	Protein	tRNA	Other RNA	Gene	Pseudogene
	master WGS	NZ_BBIY00000000.1	BBIY00000000.1	0.74	27.6	901	27	-	928	-

Genome Region

'Chrysanthemum coronarium' phytoplasma strain OY-V BBIY01000004, whole genome shotgun sequence

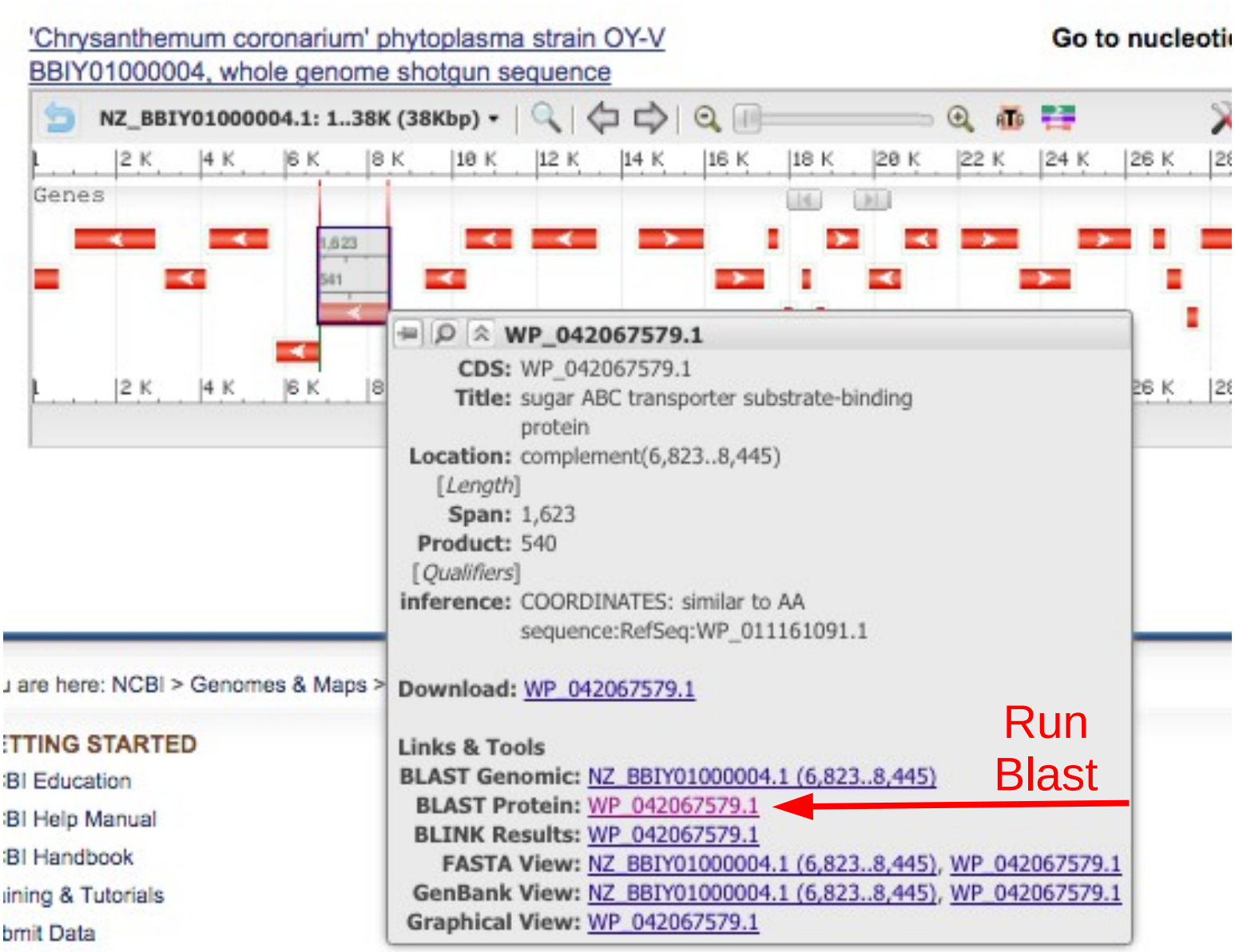
Go to nucleotide: Graphics FASTA GenBank



https://www.ncbi.nlm.nih.gov/genome/browse/

Genome Projects: Data

- Protein meta data



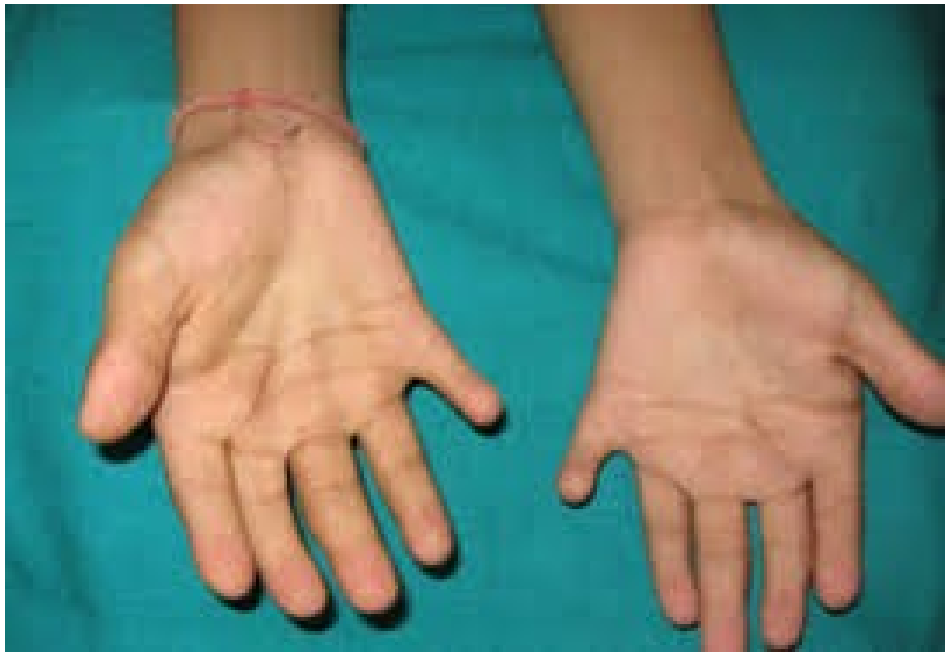
Genetic Variation

- Having diverse genetic information helps to spot genetic conditions in organisms
- Find *Genetic drift*: a random fluctuation in the population frequency of a trait
  - Occurring in descendant generations from a particular organism
  - Are evolutionary pressures causing a change in a species? Can we compare species from two different environments to learn about this drift?

Detection By Comparison
As you already know!

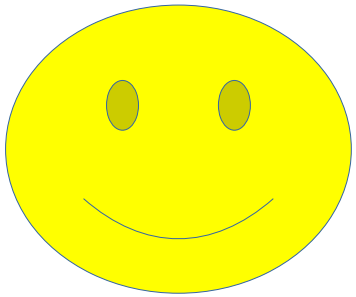
- Genetic drift may have unusual consequences.
- **How do we know where *drift* is happening?**

Hapsburg jaw



Ellis-Van Creveld
syndrome, a sixth finger

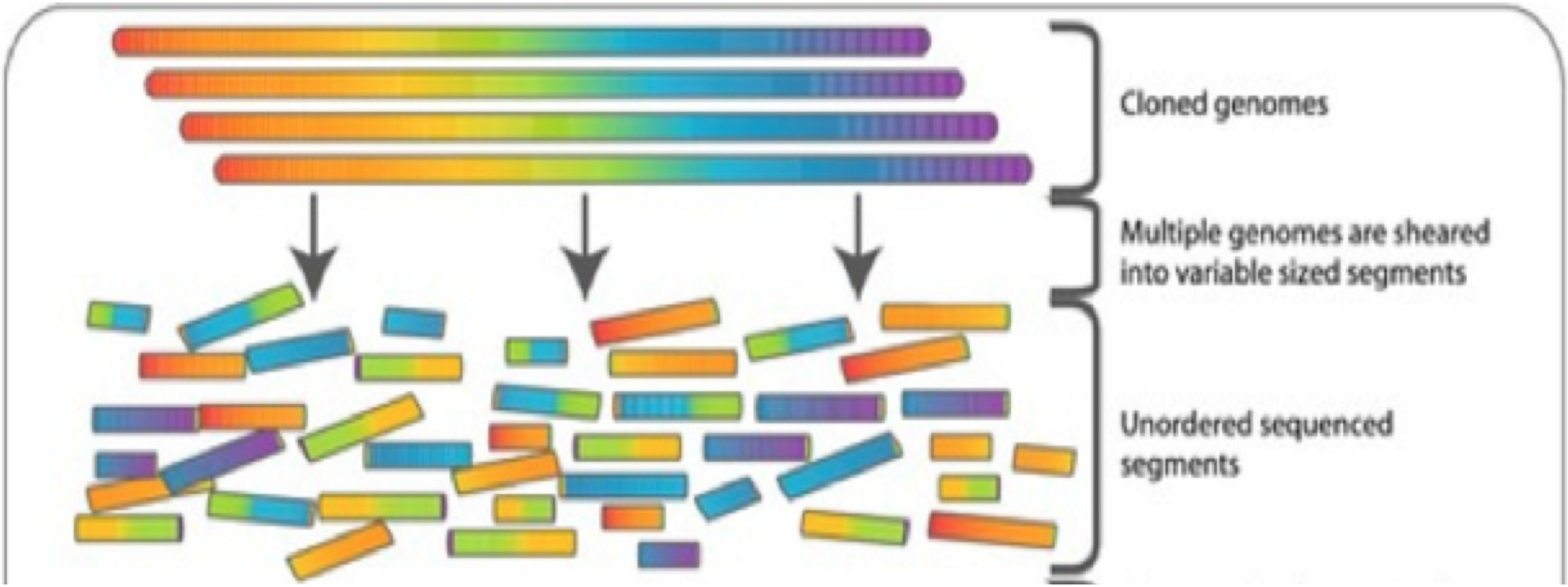
Wait! How do we get data to learn about genes??



Completing Jigsaw Puzzles

Genome Sequencing

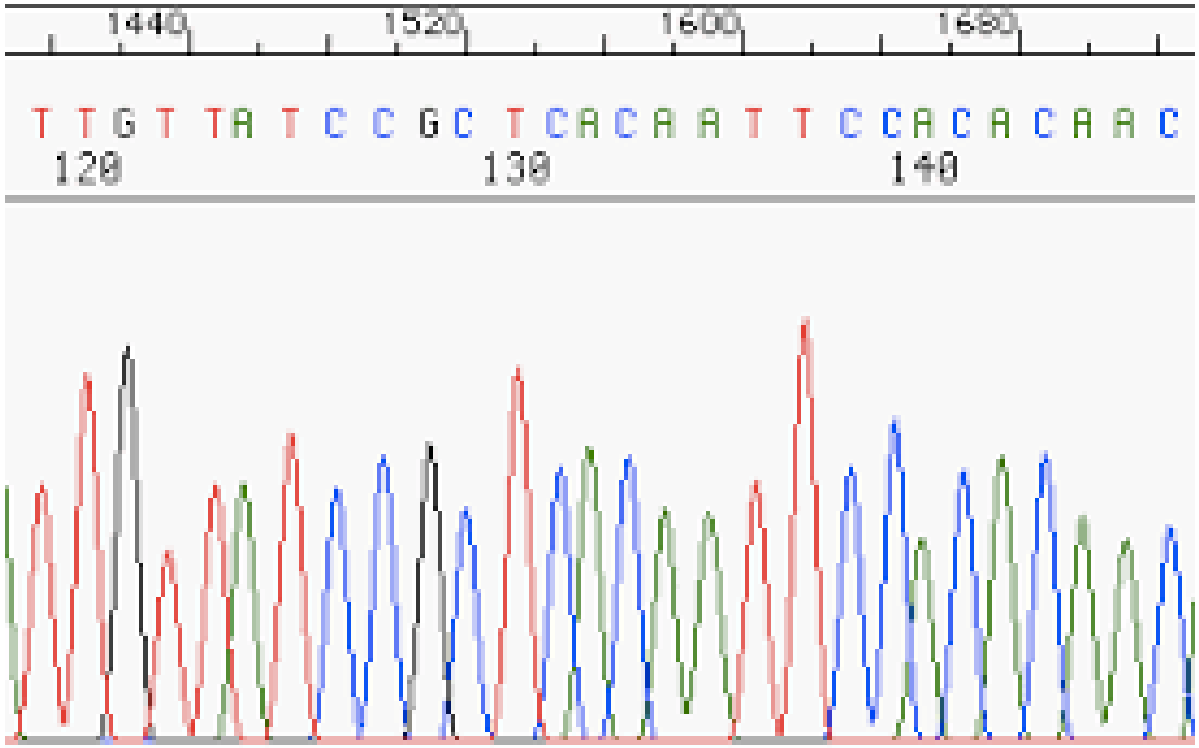
- The technology works by “exploding” DNA into smaller, manageable pieces
- It recombines pieces (***Reads***) into bigger pieces (***Contigs***)
- And then bigger chunks are combined like a jigsaw puzzle



Genome Sequencing:

Getting genetic data (for analysis)

- Bases are recorded as little peaks
- Reads = Small segments of DNA from sequencer machine
- Contigs = Segments of partially combined reads



Shredded Book Reconstruction

- Imagine that Dickens has "accidentally" shredded his first printing of a Tale of Two Cities
- What can be done to re-create the manuscript?

Shredded Book Reconstruction

- Dickens accidentally shreds first printing of Tale of Two Cities

it was the worst of

worst of times, it was…

it was the worst of

was the best of times,

It was the best of

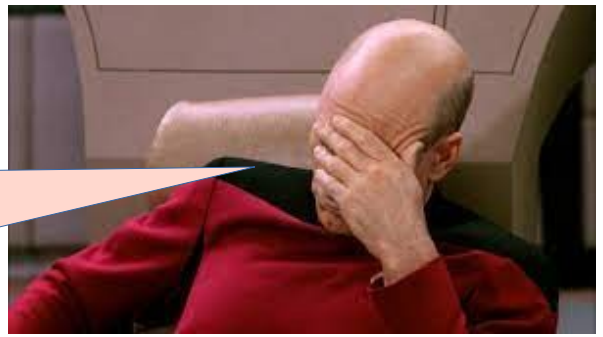
best of times, it was

age of wisdom, it was

of wisdom, it was the

wisdom, it was the age

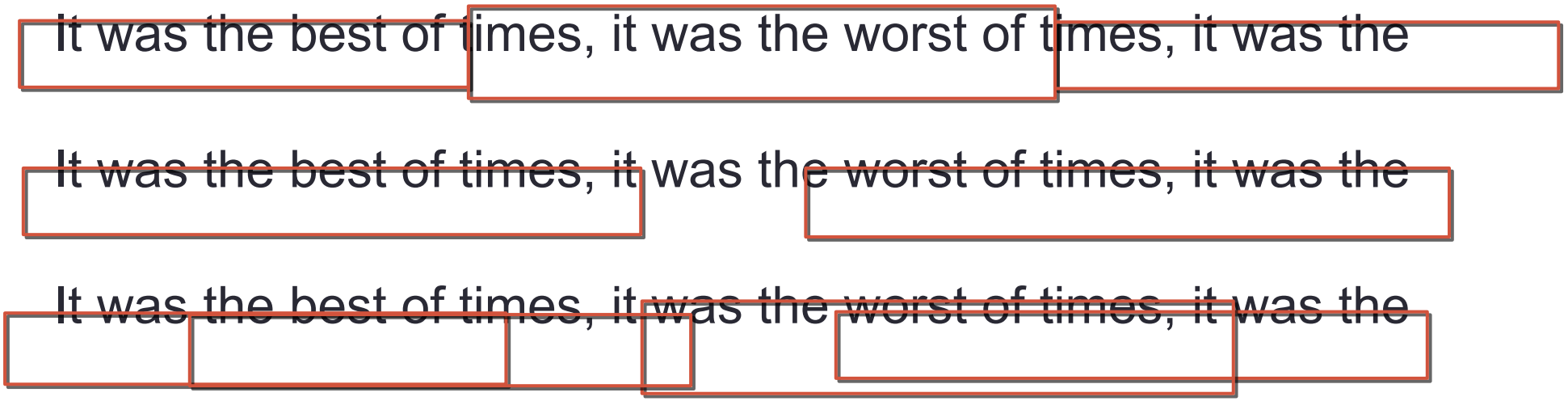
Oh no!
How do we order
these fragments back into a book!



Shredded Book Reconstruction

Dickens accidentally shreds first printing of Tale of Two Cities

- first printing = 3 copies
- shredding was random (can cut between different words in each copy)
- always 3 words per fragment



Shredded Book Reconstruction

Dickens accidentally shreds first printing of Tale of Two Cities

- first printing = 5 copies
- shredding was random (can cut between different words in each copy)
- always 5 words per fragment

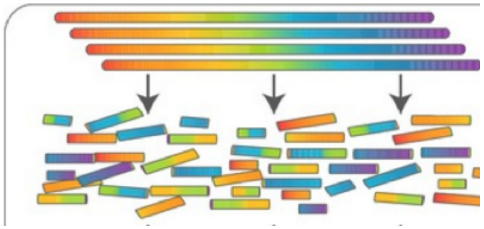
It was the best of times, it was the worst of times, it was the

It was the best of times, it was the worst of times, it was the

It was the best of times, it was the worst of times, it was the

5 copies x 138, 656 words/5 words per fragment = 138k fragments

All short fragments are mixed together



times, it was the age

It was the best of

age of wisdom, it was

best of times, it was

it was the age of

it was the age of

it was the worst of

of times, it was the

of times, it was the

of wisdom, it was the

the age of wisdom, it

the best of times, it

the worst of times, it

worst of times, it was

wisdom, it was the age

was the worst of times,

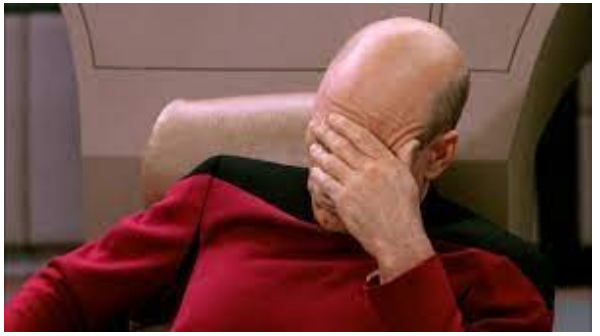
was the best of times,

was the age of foolishness,

was the age of wisdom

times, it was the worst

Oh no!
How do we order these fragments back into a sequence!



times, it was the age

It was the best of

age of wisdom, it was

best of times, it was

it was the age of

it was the age of

it was the worst of

of times, it was the

of times, it was the

of wisdom, it was the

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the worst of times, it

worst of times, it was

wisdom, it was the age

was the worst of times,

was the best of times,

was the age of foolishness,

was the age of wisdom

times, it was the worst

the best of times, it

times, it was the age

It was the best of

age of wisdom, it was

best of times, it was

it was the age of

it was the age of

it was the worst of

of times, it was the

of times, it was the

of wisdom, it was the

the age of wisdom, it

the best of times, it

the worst of times, it

worst of times, it was

wisdom, it was the age

was the worst of times,

was the best of times,

was the age of foolishness,

was the age of wisdom

times, it was the worst

the best of times, it

best of times, it was

times, it was the age

It was the best of

age of wisdom, it was

best of times, it was

it was the age of

it was the age of

it was the worst of

of times, it was the

of times, it was the

of wisdom, it was the

the age of wisdom, it

the best of times, it

the worst of times, it

worst of times, it was

wisdom, it was the age

was the worst of times,

was the best of times,

was the age of foolishness,

was the age of wisdom

times, it was the worst

Assembly Parameter: 100% identify across four words

the best of times, it

best of times, it was

times, it was the age

It was the best of

age of wisdom, it was

best of times, it was

it was the age of

it was the age of

it was the worst of

of times, it was the

of times, it was the

of wisdom, it was the

the age of wisdom, it

the best of times, it

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worst of times, it was

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times, it was the worst

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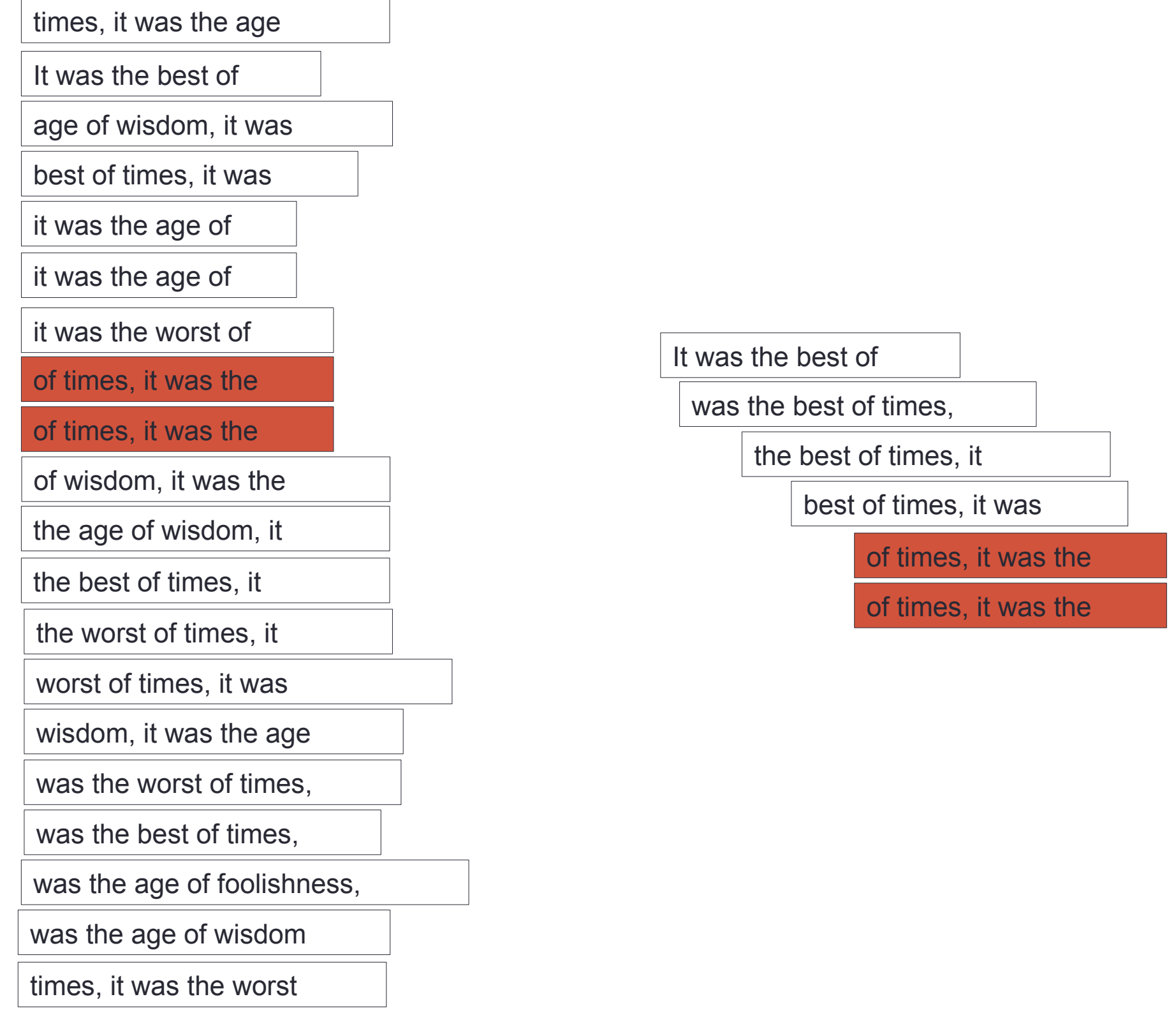

times, it was the age
It was the best of
age of wisdom, it was
best of times, it was
it was the age of
it was the age of
it was the worst of
of times, it was the
of times, it was the
of wisdom, it was the
the age of wisdom, it
the best of times, it
the worst of times, it
worst of times, it was
wisdom, it was the age
was the worst of times,
was the best of times,
was the age of foolishness,
was the age of wisdom
times, it was the worst
It was the best of
was the best of times,
the best of times, it
best of times, it was
of times, it was the
of times, it was the

Tale of Two Cities
Charles Dickens

It was the best of times, it was the worst of times, it was the age of wisdom, it was the age of foolishness, it was the epoch of belief, it was the epoch of incredulity, it was the season of Light, it was the season of Darkness, it was the spring of hope, it was the winter of despair, we had everything before us, we had nothing before us, we were all going direct to Heaven, we were all going direct the other way - in short, the period was so far like the present period, that some of its noisiest authorities insisted on its being received, for good or for evil, in the superlative degree of comparison only.

Making sense of it all:
We can already see how these words are coming together!

times, it was the age

It was the best of

age of wisdom, it was

best of times, it was

it was the age of

it was the age of

it was the worst of

of times, it was the

of times, it was the

of wisdom, it was the

the age of wisdom, it

the best of times, it

the worst of times, it

worst of times, it was

wisdom, it was the age

was the worst of times,

was the best of times,

was the age of foolishness,

was the age of wisdom

times, it was the worst

It was the best of

was the best of times,

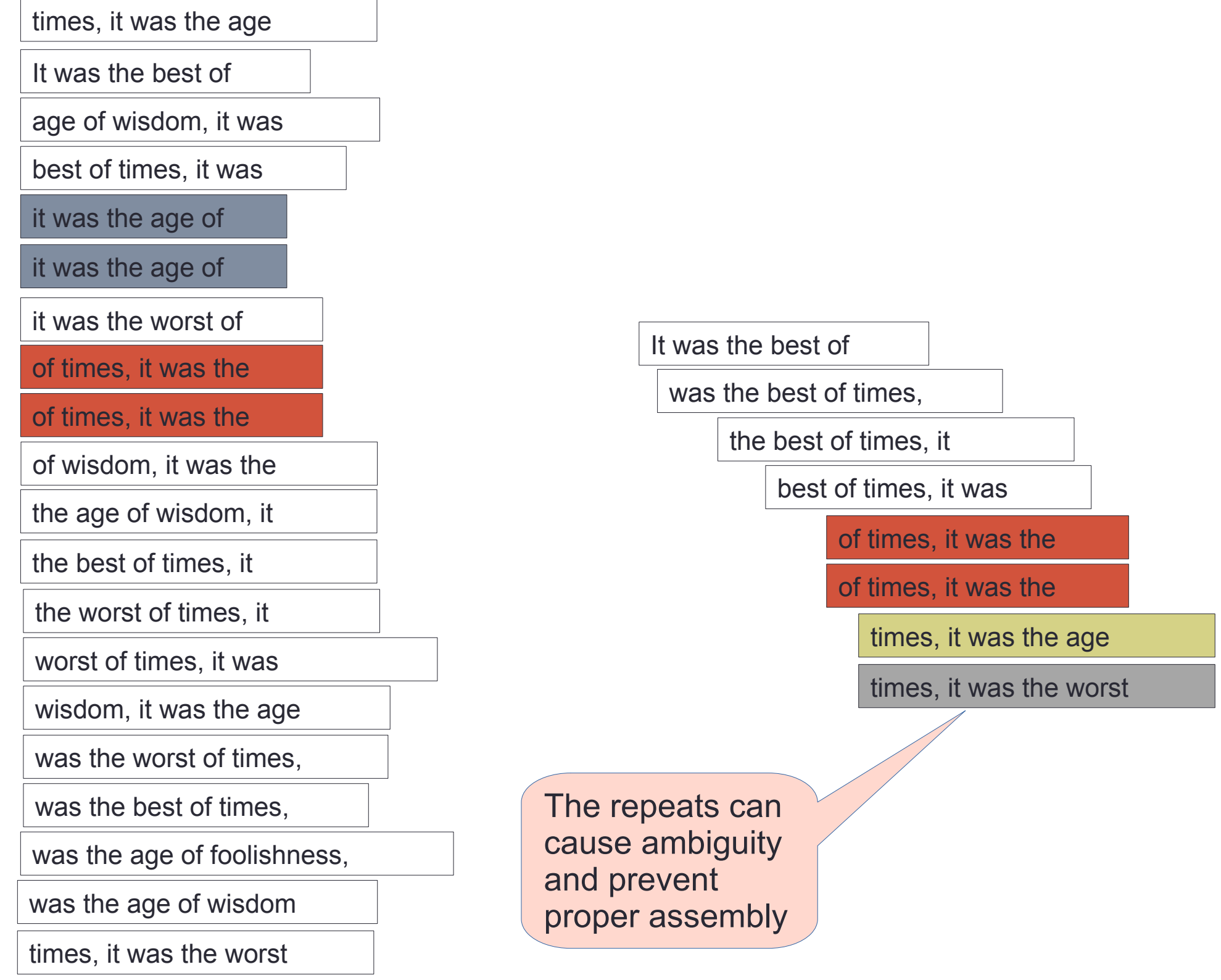
the best of times, it

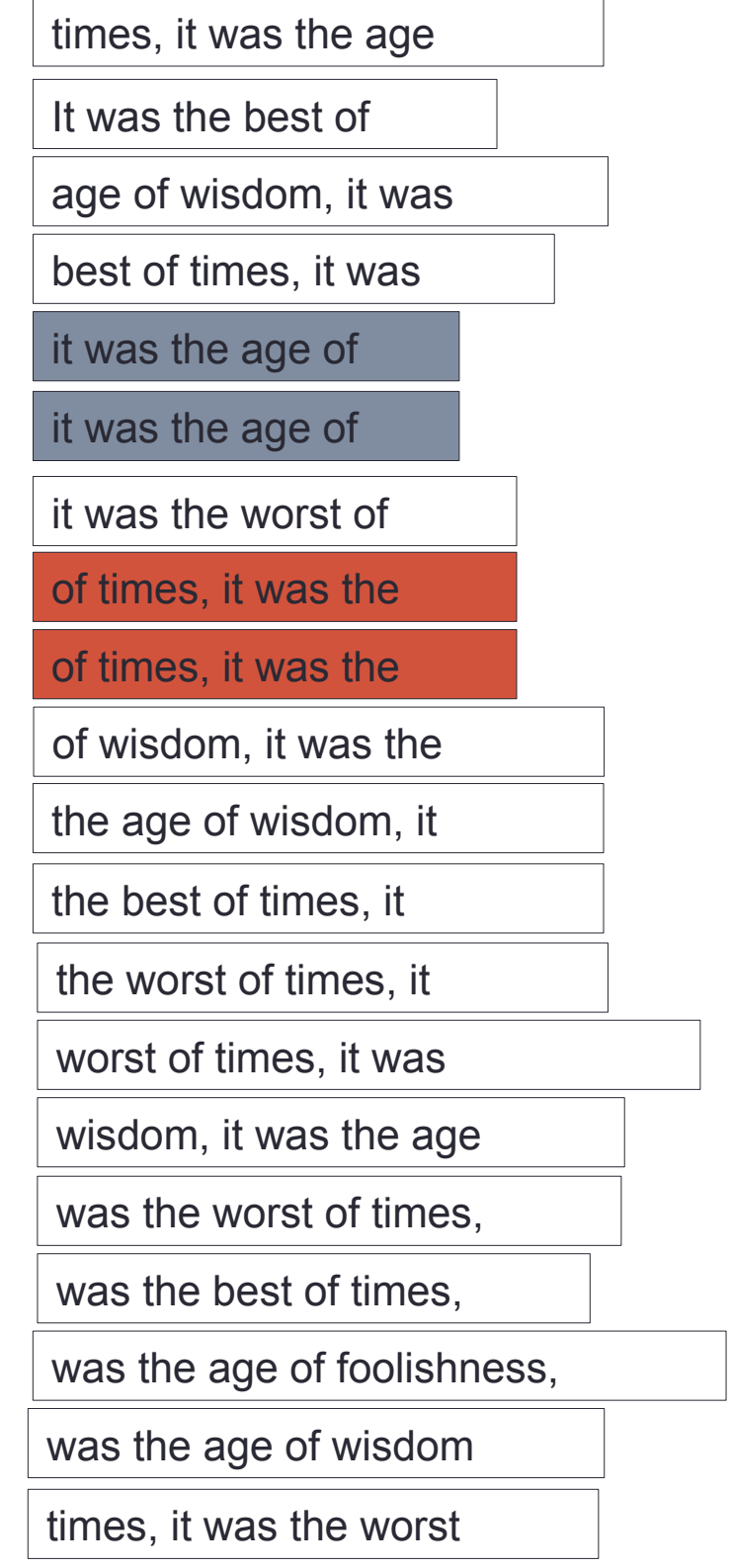
best of times, it was

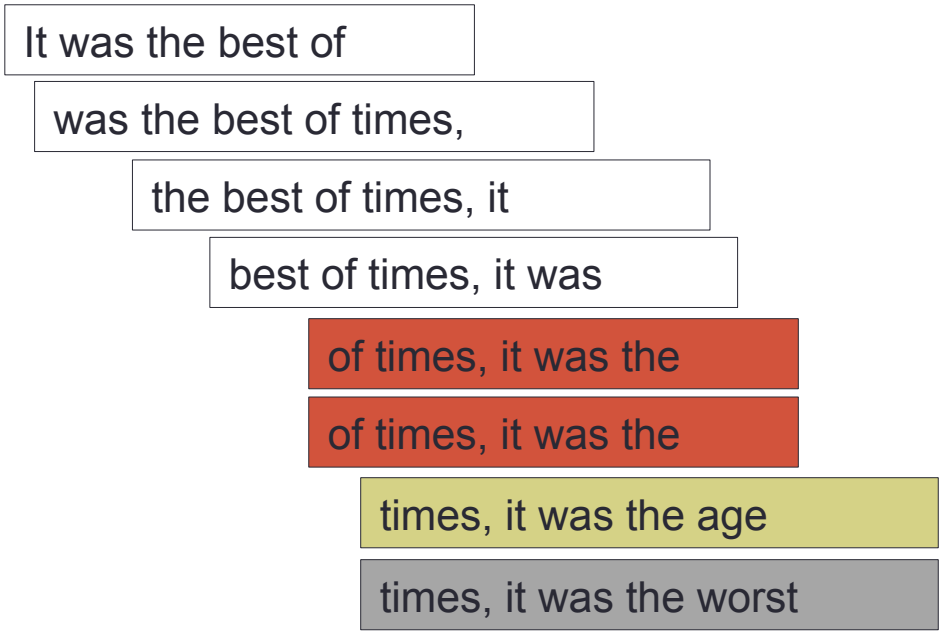
of times, it was the

of times, it was the

The *repeats* pile up:
The actual placement of each individual fragment unknown


times, it was the age
It was the best of
age of wisdom, it was
best of times, it was
it was the age of
it was the age of
it was the worst of
of times, it was the
of times, it was the
of wisdom, it was the
the age of wisdom, it
the best of times, it
the worst of times, it
worst of times, it was
wisdom, it was the age
was the worst of times,
was the best of times,
was the age of foolishness,
was the age of wisdom
times, it was the worst
It was the best of
was the best of times,
the best of times, it
best of times, it was
of times, it was the
of times, it was the
times, it was the age
times, it was the worst
The repeats can cause ambiguity and prevent proper assembly

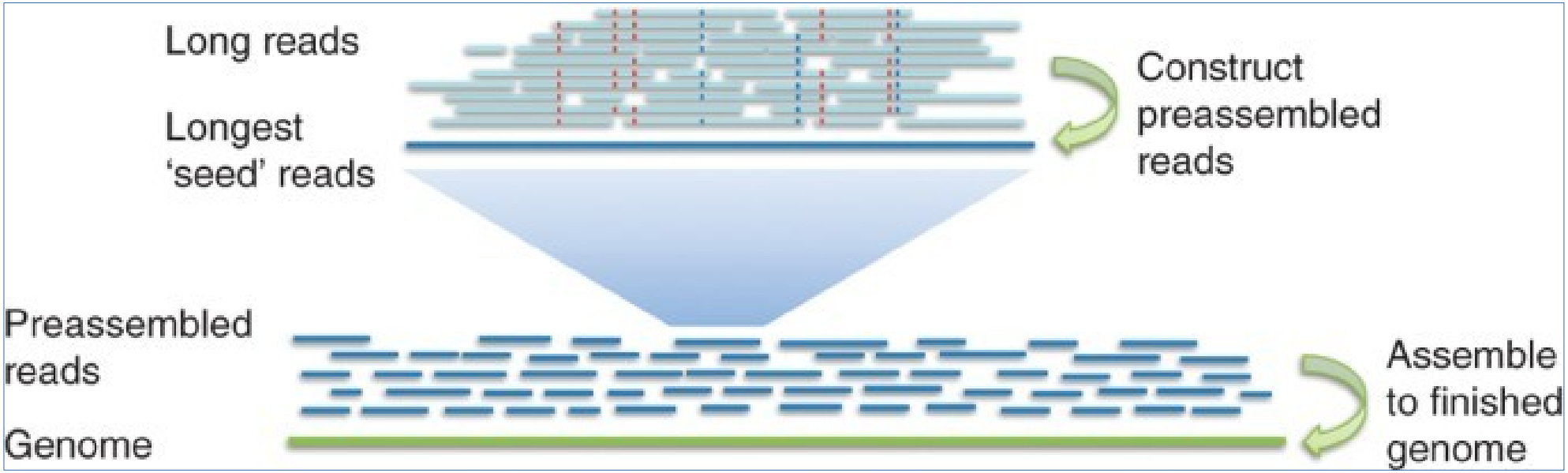


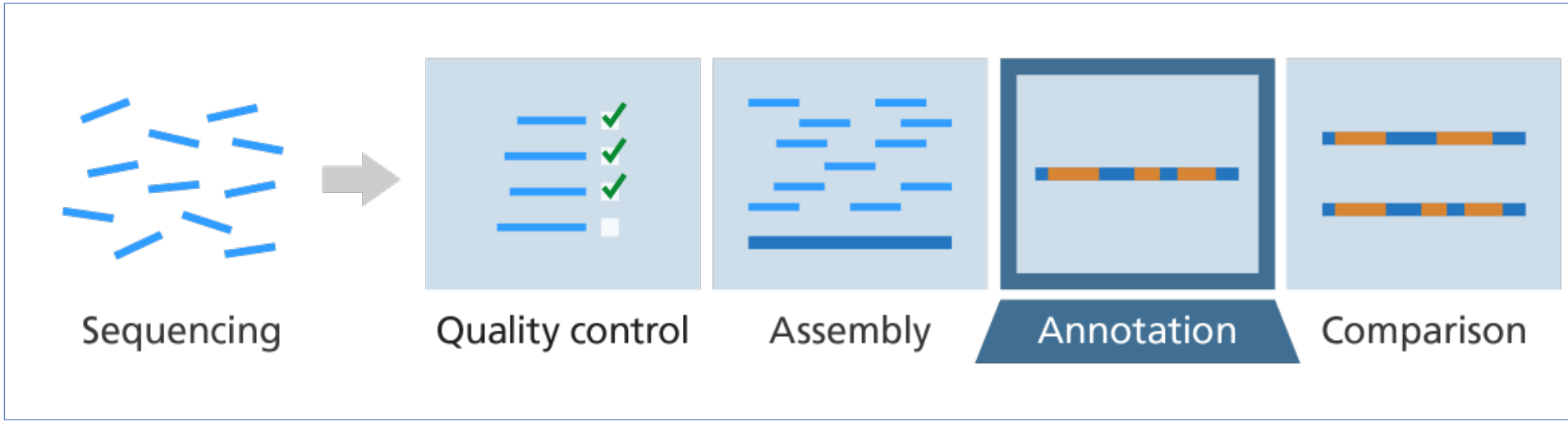


It was the best of times, it was the [age/worst]

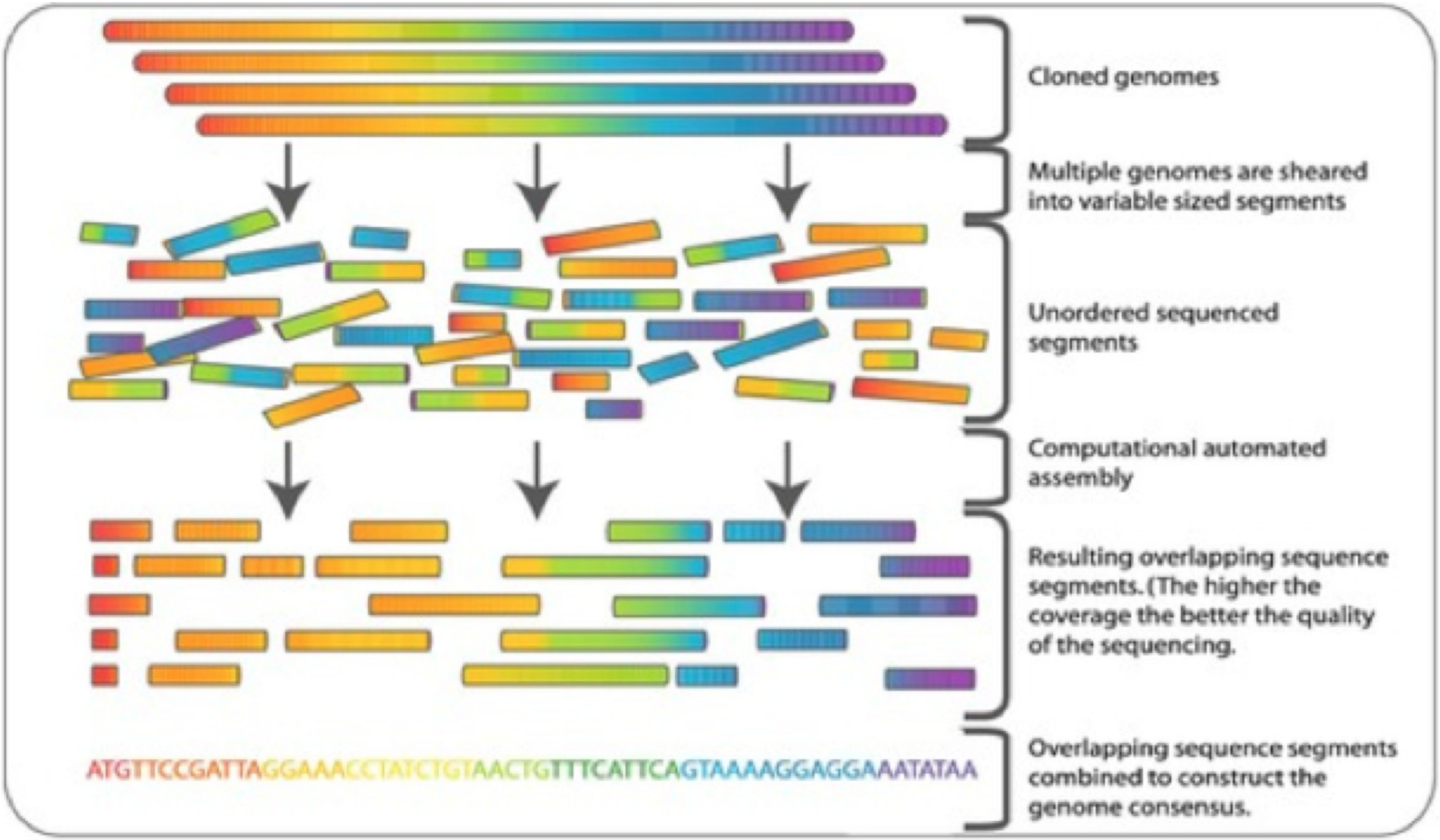
Which word to use here?!

Overview


Long reads
Longest 'seed' reads
Construct preassembled reads
Preassembled reads
Genome
Assemble to finished genome

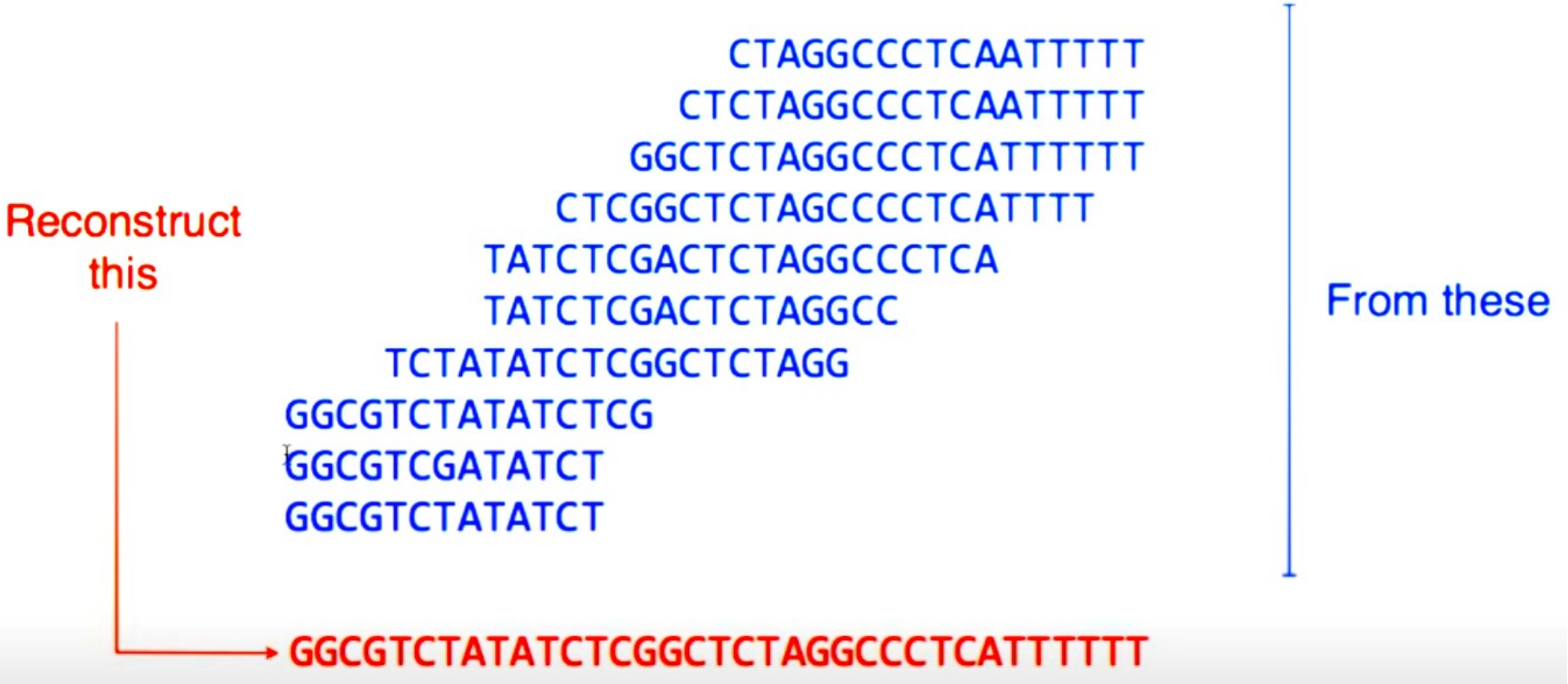

Sequencing
Quality control
Assembly
Annotation
Comparison

Sorting Reads to Make Contigs

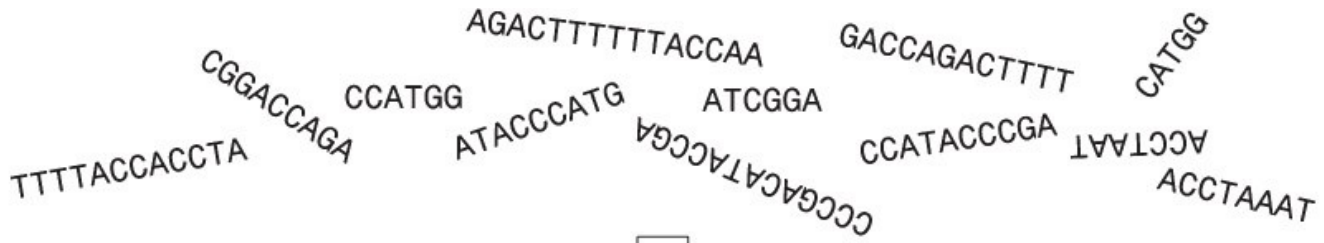


Fragments of Genes

Assume sequencing produces such a large # fragments that almost all genome positions are *covered* by many fragments...



Coverage and Ordering



random short sequence reads

1122332233222332222223222222222333332221222234433 2

coverage

AGACTTTTTTACCAA CCATACCCGA CCATGG
ATCGGA TTTTACCAACCTA CCCGACATACCGA
GACCAGACTTTT ACCTAAAT ATACC CATGG
CGGACCAGA AATCCATA ATACCCATG

assembly of overlapping fragments

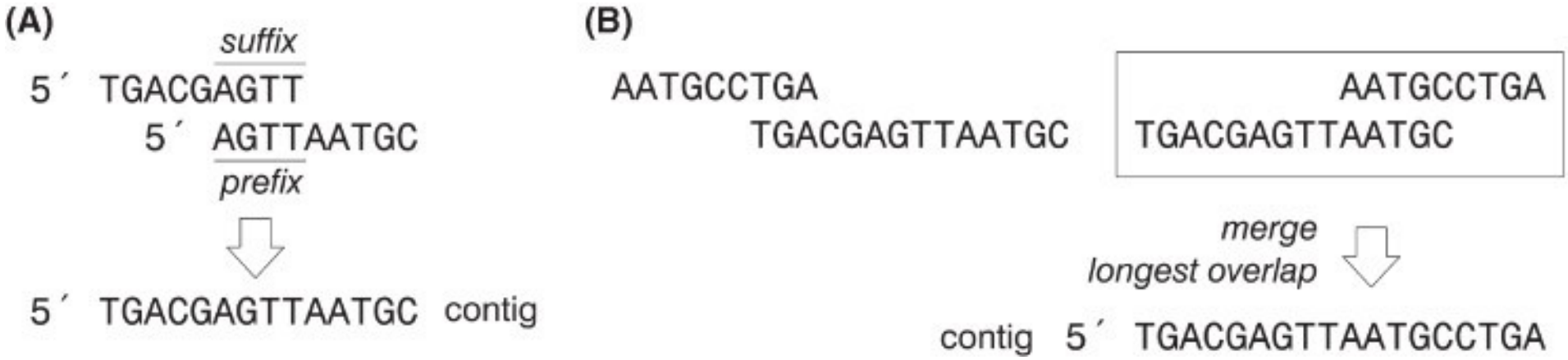
ATCGGACCAGACTTTTTTACCAACCTAAATCCATACCCGACATACCCATGG

assembled contig sequence

Finding the Largest Overlap

- Consider the assembly of two fragments:
  - If there is more than one overlap, choose the **longest** overlap
  - Assume the sequences are not identical
  - Assume neither sequence is a substring of the other
  - The longest **possible** overlap is length of the shorter sequence minus a character (to determine placement in the larger sequence)

Algorithm to Find Overlaps



1. Start with reads; s1 and s2
2. *n* = size of the smallest sequence – 1
3. Compare *n* suffix/prefix characters from s1 with *n* prefix/suffix characters s2
4. Count matching bases in the prospective overlap region. If the number of matches = *n*, then the largest overlap is found
5. If the number of matches < *n*, *n* = *n*-1
6. If *n* = 0 then no overlap, go to step 3

Noting Assembling Contigs

Table 8.3 Overlaps for a hypothetical set of sequence reads.

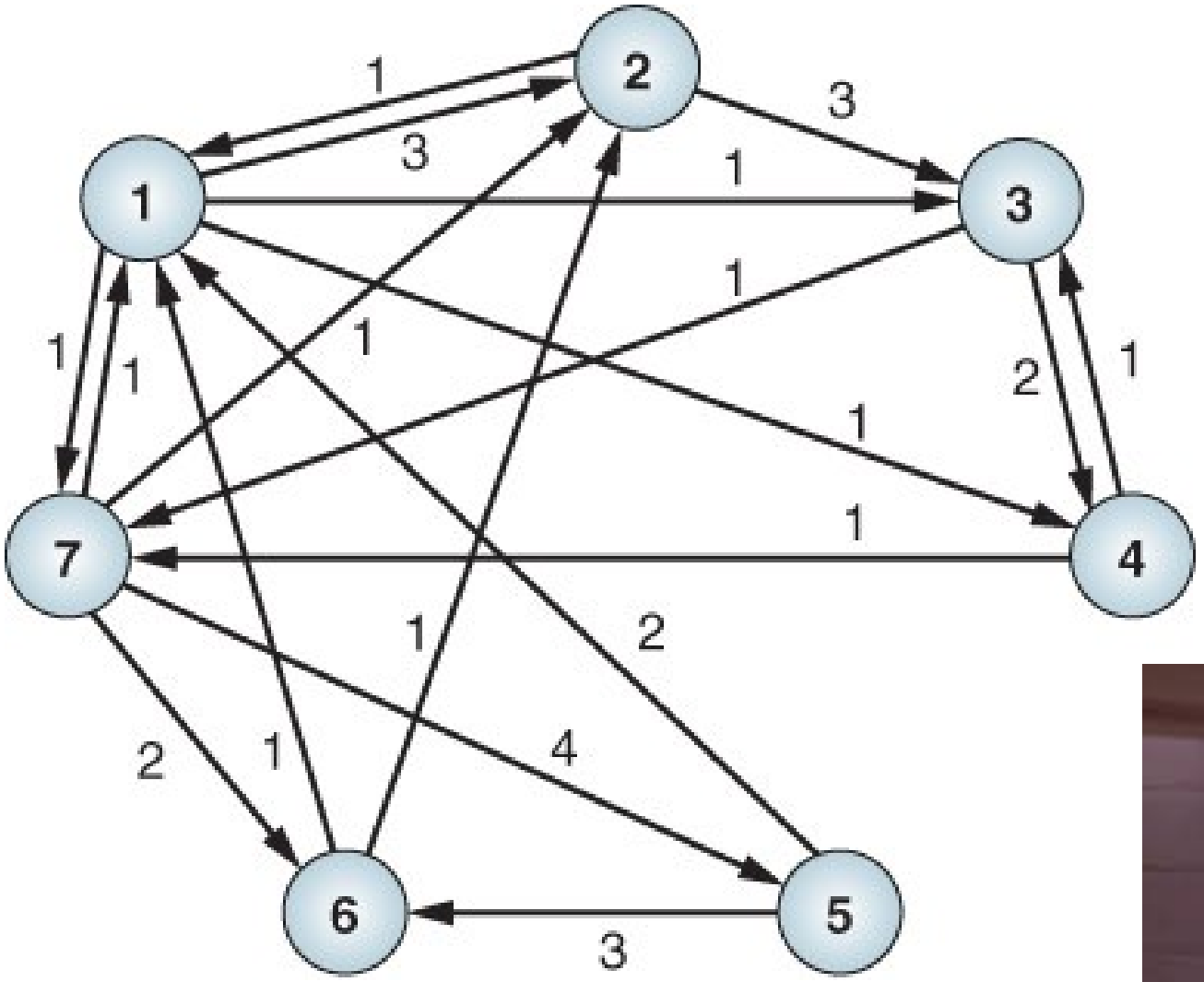
Fragments	Overlaps (Length)
1. TACCTTG	2 (3), 3 (1), 4 (1), 7 (1)
2. TTGAT	1 (1), 3 (3)
3. GATATGG	4 (2), 7 (1)
4. GGAG	3 (1), 7 (1)
5. CTCTA	1 (2), 6 (3)
6. CTAGT	1 (1), 2 (1)
7. GCTCT	1 (1), 2 (1), 5 (4), 6 (2)

For each sequence, we name an overlap with another sequence by number and number of overlaps.

```
Seq1:  TACCTTG
Seq2:     TTGAT
```

With_Seq (num of overlaps)
Ex: 2 (3)
Seq 1 has three overlaps with Seq 2

Assembling a Contig: graph representation

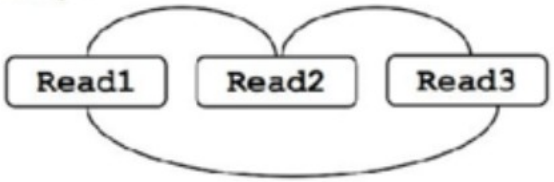


Make It so!

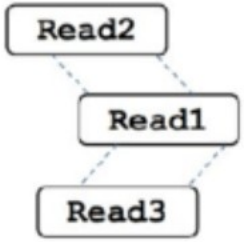
Two Basic Techniques

(a) Overlap, Layout, Consensus assembly

(i) Find overlaps



(ii) Layout reads



(iii) Build consensus

```
CGATTCTA
 TTCTAAGT
GATTGTAA
CGATTCTAAGT
```

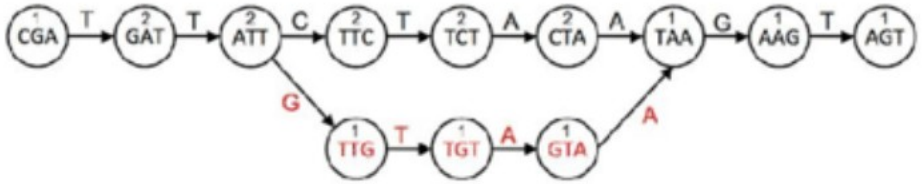
We just saw this one

(b) De Bruijn graph assembly

(i) Make kmers

```
Read1: TTCTAAGT   Read2: CGATTCTA   Read3: GATTGTAA
Kmers: TTC        Kmers: CGA        Kmers: GAT
       TCT               GAT               ATT
       CTA               ATT               TTG
       TAA               TTC               TGT
       AAG               TCT               GTA
       AGT               CTA               TAA
```

(ii) Build graph



(iii) Walk graph and output contigs

CGATTCTAAGT

Same idea but we use *k*-mers here